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ORIGINAL ARTICLE

Microbial Quality Evaluation and Prevalence of Bacteria and Fungi in Different Varieties of Chicken Meat in Lahore

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ABSTRACT

Objective: This study was conducted to evaluate the bacterial and fungal contamination in the chicken meat.

Method: For this purpose, a total of 180 samples of different categories of the chicken meat were collected from the areas of Kot Radha Kishan, Raiwind and Lahore city from which 9 types of bacterial species and 8 types of fungal species were isolated and confirmed by microscopy and biochemical analysis.

Results: The Mean Viable Count (MVC) of the raw unprocessed (RU), raw processed (RP) and cooked processed (CP) chicken meat were 7.9log10CFU/g, 4.37log10CFU/g and 2.66log10CFU/g respectively (p-value < 0.05) that lie in the unacceptable range for the human consumption. In RU meat, Staphylococcus epidermidis and Shigella sonnei were more prevalent while RP meat had the highest load of Bacillus cereus. Similarly, in CP meat, Bacillus cereus was more prevalent. It was also found that Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus were the most prevalent fungi in RU, RP and CP meat respectively while Fusarium avenaceum was only isolated from the RP meat.

Conclusion: It was concluded from the study that hygiene of the slaughtering facility of the open market and the personal hygiene of workers of the processing plants was very poor and the chicken meat of this study area was unsafe for use.

INTRODUCTION

Pakistan poultry industry has increased its production rate from 20% to 25% per annum producing meat 0.652 million tons that is 23% of the total meat production of the country [1]. Further processing and development of new products has increased the demand of chicken meat [2].

There is an association between the incidence of outbreaks of foodborne illness and consumption of the poultry meat [3,4]. In most of the countries, poultry and poultry products are ranked top foods to be associated with the diseases [5].

In spite of the hygienic slaughtering and modern processing techniques, food safety has been the major public health issue [6]. The basic parameter for consumers, producers, and public health officials is the safety of the processed poultry products especially for those products that are highly contaminated [7]. These unwanted microorganisms are undesirable during the processing, handling, and transportation of the chicken meat. There is an expectation of higher bacterial load on the carcass

when handled unhygienically [8]. In India, 5% of the chicken meat products was produced by processing units while rest of the chicken meat was produced by slaughtering the birds in an un-organized sector (retail shops) where there is a high risk of contamination due to poor hygienic conditions [9].

Two main pathogens causing foodborne illnesses are Staphylococcus aureus and Bacillus cereus. The most occurring outbreaks of Staphylococcal and B. cereus food poisoning may be due to extensively handled and improperly cooked meat products [10].

It is suggested that yeast and molds play an important role in meat spoilage [11,12]. Fungal contaminations in food is very useful indicator to evaluate the quality of food [13]. Fungi commonly contaminate meat and its products by causing spoilage with producing mycotoxins [14] which further damages liver, cause liver cancer and food poisoning in humans [15,16].

METHODOLOGY

Collection of samples

A total of 180 chicken meat samples comprising of three main varieties; RU, RP and CP which were further categorized into thigh (T) and breast (B) parts of the meat in raw varieties and Nuggets (N), Tender pops (TP), Harey Bharey nuggets (HBN) and Chicken Tempura (CT) as processed meat samples were purchased from the retail shops of Raiwind, Kot Radha Kishan and Lahore city, Pakistan. Packed separately in the sterile polyethene bags aseptically, they were transported to the laboratory of Microbiology of the Institute of Molecular Biology and Biotechnology, The University of Lahore immediately for analysis.

Preparation of sample

Samples were prepared according to the set method [17]. A part of the meat was cut with sterile knife by wearing sterile plastic gloves and 25g of meat was separately grounded in a sterile mechanical blender, cleaned and disinfected in between samples to prevent cross-contamination and then mixed with 225ml of sterile buffered peptone water (0.1%). After homogenizing it, one milliliter of the homogenate was introduced into the test tube having 9ml buffered peptone water, labeled 1:10 (10-1) dilution and then serially diluted into nine more test tubes, labeled 10-2, 10-3, 10-4, 10-5, 10-6, 10-7, 10-8, 10-9 and 10-10.

Preparation of media

All the biochemical media were prepared by following their respective method for further processing of the sample.

Culturing of sample and bacterial count

From each dilution of each diluted sample, 100ml was spread over the Nutrient Agar plate and Sabouraud's Dextrose Agar plate for aerobic incubation at the temperature of 37°C for 24-48 hours. After the incubation total plate count (TPC) was calculated with the help of digital colony counter.

Purification of isolated colonies

All the discrete colonies obtained from nutrient agar were then isolated on specific media which included Salmonella/Shigella Agar, Mannitol Salt Agar, Eosin Methylene Blue Agar, Cetrimide Agar supplemented with Glycerol, Blood Agar Base supplemented with 5% (v/v) Sheep blood and Bacillus Cereus Agar Base supplemented with Polymyxin B and Egg yolk. Incubation was set 37°C for 24 hours to obtain the growth cultures. Furthermore, pure cultures were obtained by streaking a portion of the isolated colonies from the specific media onto the nutrient agar and aerobically incubated at 37°C for 24 hours [18].

Microscopy

Bacterial cell morphology was examined under light microscope after using Gram´s staining, Capsule staining and Spore staining.

Biochemical test

The pure bacterial isolates were confirmed by biochemical tests including Catalase, Oxidase, Urease, Coagulase, VP reaction, Citrate, Indole, Methyl red, NO_3 reduction, H_2S production, Gelatin liquefaction and Starch hydrolysis.

Preservation of isolated strains

The isolated bacteria were preserved in 40% Nutrient broth with 60% Normal Saline in 1.5ml Eppendorf tube and frozen at -18°C for further use.

The results of the samples were compared through One Way ANOVA test for each variety using SPSS v16.0 to determine difference in group means at P value ≤ 0.05.

RESULTS

Bacterial load

Bacterial load was counted as a log10CFU/g. In the categorical analysis of the chicken meat it was found that RU chicken meat had highest average bacterial load of 7.9log10CFU/g while RP meat had less load of 4.37log10CFU/g and the CP meat had least average bacterial load of 2.66log10CFU/g which concluded that cooked/ processed category was least contaminated from bacteria than raw categories. The analysis of each variety showed that thigh meat variety had more bacterial load than breast meat varieties, chicken tempura had highest bacterial load while tender pops had the least. It also showed that all the four cooked varieties were less contaminated from bacteria than raw varieties which is shown in the Figure **1.**

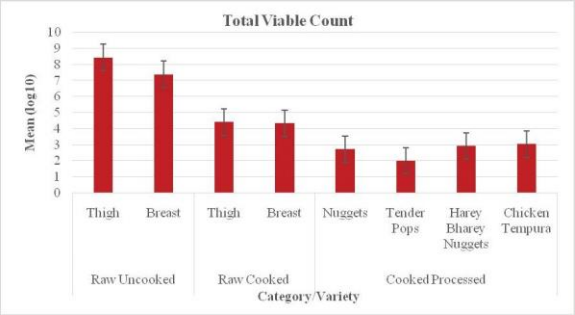

Figure 1. Bacterial load in the chicken meat samples.

Biochemical characterization

Twelve different biochemical tests were performed on the isolated bacteria which reconfirmed the suspected strains. Details of the tests performed and confirmed bacteria are shown in Table **1**.

Microscopy

After using multiple staining techniques, (Gram's stain, Capsular stain and Spore stain) 5 strains were observed as Gram positive and 4 as Gram negative among all stains obtained from initial samples.

Prevalence of isolated bacteria

Prevalence of bacteria in each category showed the amount of contamination in that category. It was found that in RU meat, prevalence of S. epidermidis and S. sonnei were the highest 50% of both followed by S. enterica 41.6% while E. faecalis found least prevalent 28.3%. RP meat had highest load of B. cereus 35% followed by E. faecalis 25% while least load of S. aureus 13.3%. Similarly, in CP meat, B. cereus 30% followed by E. faecalis 21.6% were more prevalent and least prevalent was S. epidermidis 8.3% as shown in the Table **2**.

Prevalence of fungal isolates

The prevalence of 8 isolated fungi in different categories of the samples showed the fungal contamination in that category of the chicken meat. It was found that in RU meat, A. niger (23.3%), in RP meat, A. flavus (20%) and in A. fumigatus (10%) were the most prevalent molds while in RP meat, R. stolonifer (6.6%) was least prevalent than other molds. It was also found that F. avenaceum (10%) was only isolated from the RP meat while absent in other categories.

Yeasts isolated from each category were more in numbers as compare to the molds but it was less prevalent (28.3%) in RP meat than other categories as shown in Table **3**.

Table 1. Biochemical confirmation of isolated bacteria.

Bacterial Isolates	Catalase	Oxidase	Urease	Citrate	VP reaction	Methyl Red	Indole	NO_3 Reduction	H_2S Production	Starch Hydrolysis	Gelatin Liquefaction	Coagulase
S. aureus	+	-	+	-	+	+	-	+	-	-	+	+
S. epidermidis	+	-	+	-	+	-	-	+	+	-	-	-
S. enterica	+	-	-	+	-	+	-	+	+	-	-	
S. sonnei	+	-	-	-	-	+	-	+	-	-	-	
P. aeruginosa	+	+	-	+	-	-	-	+	-	-	+	
E. coli	+	-	-	-	-	+	+	+	-	-	-	

B. cereus	+	-	-	-	+	-	-	+	-	+	+	
E. faecalis	-	-	-	-	+	-	-	-	-	+	+	
M. luteus	+	-	+	-	-	-	-	+	-	-	+	

Table 2. Prevalence of isolated bacteria in each category of chicken meat.

Bacterial Isolates	T(n=30)	Raw Unprocessed B(n=30)	Percentage (%)	T(n=30)	Raw Processed T(n=30)	T(n=30)	N(n=15)	Cooked Processed TP(n=15)	HBN(n=15)	CT(n=15)	Percentage (%)
S. aureus	16	8	40	4	4	13.3	2	1	2	2	11.6
S. epidermidis	19	11	50	5	4	15	2	0	1	2	8.3
S. enterica	13	12	41.6	7	3	16.6	2	2	2	3	15
S. sonnei	16	14	50	8	5	21.6	3	2	3	3	18.3
P. aeruginosa	14	9	38.3	7	3	16.6	1	3	2	2	13.3
E. coli	13	9	36.6	6	3	15	1	2	3	1	11.6
B. cereus	9	11	33.3	14	7	35	3	4	5	6	30
E. faecalis	9	8	28.3	7	8	25	3	3	2	5	21.6
M. luteus	9	10	31.6	5	4	15	1	2	2	2	11.6

T = Thigh, B = Breast, N = Nuggets, TP = Tender Pops, HBN = Harey Bharey Nuggets, CT = Chicken Tempura

Table 3. Prevalence of fungal isolates in different categories of chicken meat.

Fungal Isolates	RU(n=60)	Percentage (%)	RP(n=60)	Percentage (%)	CP(n=60)	Percentage (%)
A. niger	14	23.3	10	16.6	5	8.3
A. fumigatus	10	16.6	10	16.6	6	10
A. flavus	7	11.6	12	20	4	6.6
P. chrysogenum	9	15	8	13.3	5	8.3
R. stolonifer	11	18.3	4	6.6	3	5
F. equiseti	10	16.6	6	10	1	1.6
F. avenaceum	0	0	6	10	0	0
Yeast	26	43.3	17	28.3	26	43.3

RU = Raw Unprocessed, RP = Raw Processed, CP = Cooked Processed

DISCUSSION

There is an association between microbes and food we eat [20]. Microorganisms introduced during the processing steps may come from normal micro flora or from the environment affect the food, can spoil the food and may cause food borne illnesses.

In the present studies, Mean Viable Counts (MVC) of RU, RP and CP were 7.9 log10CFU/g, 4.37log10CFU/g, and 2.66log10CFU/g respectively. The Mean Viable Count of the raw thigh and the

breast meat was found to be 8.43log10CFU/g and 7.38log10CFU/g respectively. Similarly, the Mean Viable Count of the raw processed thigh meat was 4.41log10CFU/g as compared to the breast meat (4.33log10CFU/g). In the case of cooked processed meat, chicken tempura variety had comparatively higher mean viable count (3.03log10CFU/g), followed by Harey Bharey nuggets (2.92log10CFU/g), nuggets (2.71log10CFU/g) and tender pops (1.99log10CFU/g). The recommended limit of bacterial contamination for foods by International microbiological standards is 105 cfu/g for total bacterial plate count [21,22].

Contrary to the findings of the present studies, the leg parts of chicken collected from the freezer depots, from open market, and from cold room had total plate count 1.4×102 cfu/g, 1.5×103 cfu/g and 1.5×101 cfu/g respectively [23].

Eman et al., 2012A similar study reported that from cooked chicken product samples, APC of Luncheon and Shawerma were 8.5×103 and 1.2×105 respectively. They also reported that APC for E. coli were 3.7×10 in Luncheon and 3.9×102 in Shawerma and for S. aureus were 1×103 in Luncheon and zero in Shawerma [24] that is contrary to the present study.

All the chicken parts collected from different sources, have a very high microbial load and thus microbiologically unacceptable for consumption. The high microbial flora might have been as a result of poor hygiene, poor sanitary conditions of the tables, spores of bacteria in the environment, knives, flies and poor hygienic conditions of the workers and improper maintenance of the cold chain.

In the present studies, the most prevalent bacteria were Bacillus cereus isolated from 32.7% samples followed by Shigella sonnei from 30%, Entrococcus faecalis from 25%, Staphylococcus epidermidis from 24.4%, Salmonella enterica from 24.4%, Pseudomonas aeruginosa from 22.7%, Staphylococcus aureus from 21.6%, Escherichia coli from 21.1% and the least prevalent was Micrococcus luteus isolated from 19.4% samples.

The incidence of Salmonella in chicken carcasses ranged from 20% to 70% in most of the countries [25]. Contrary to the findings of the present study, the prevalence of Salmonella in chicken carcasses was higher 57% reported in Northern Thailand [26], 69% in India [27], 38% in Pakistan [28] and 36% and 14.5% in Malaysia in two respective studies [29,30]. This study is in agreement with those observed in Belgium 23%-34% [31] and United Kingdom 25% [32]. The reason of this high rate of salmonella could be the lack of proper cold chains, unhygienic conditions and inadequate power supply in retail outlets [33].

In different studies, prevalence of Staphylococcus ranged from 82% to 100% [34], 90% [35] and 95% [36] reported in market samples of chicken meat that were contrary to the findings of the present study. Presence of staphylococcus in food indicates human contact such as poor personal hygiene and poor manufacturing practices [37]. Enterotoxins produced by Staphylococcus, can bear high temperature and can cause vomiting and diarrhea on ingestion [38]. They can also tolerate high sodium chloride concentration [39]. Staphylococcal food poisoning rarely cause death but only in small children and immunocompromised persons [40].

Contrary to the findings of the present study, the prevalence of E. coli in chicken meat ranged from 42% to 88% and the thigh muscle were more contaminated than breast muscle [34] and same contrary result was also reported [41]. Prevalence of E. coli in Luncheon and Shawerma were 25% and 20% respectively and that of Staphylococcus aureus were 10% and zero respectively [24] that is in agreement with the present study.

The prevalence of S. enterica in the category of RU, RP and CP chicken meat were 41.6%, 16.6% and 15% respectively. Concurrence with the findings of the present study, prevalence of Salmonella species in chicken breast muscle from non-sophisticated outlets, moderate facility, sophisticated outlets and poultry processing facility were 65.7%, 48.55, 48.5% and 22.8% respectively and that of thigh muscle were 71.4%, 51.45, 48.5% and 25.7% respectively which showed that contamination of salmonella in meat decreased with increase in sophistication of slaughter facility [34].

Bacteriological analysis of each variety of the chicken meat showed that thigh muscle of RU meat prevalence of S. aureus, S. epidermidis, S. enterica, S. sonnei, P. aeruginosa, E. coli, B. cereus, E. faecalis and M. luteus were 53.3%, 63.3%, 43.3%, 53.3%, 46.6%, 43.3%, 30%, 30% and 30% respectively; from breast muscle of RU meat were 26.6%, 36.6%, 40%, 46.6%, 30%, 30%, 36.6%, 26.6% and 33.3% respectively; from thigh muscle of RP meat were 13.3%, 16.6%, 23.3%, 26.6%, 23.3%, 20%, 46.6%, 23.3% and 16.6% respectively; from breast muscle of RP meat were 13.3%, 13.3%, 10%,

16.6%, 10%, 10%, 23.3%, 26.6% and 13.3% respectively; from nuggets of CP meat were 13.3%, 13.3%, 13.3%, 20%, 6.6%, 6.6%, 20%, 20%, 6.6% respectively; from tender pops of CP meat were 6.6%, 0%, 13.3%, 13.3%, 20%, 13.3%, 26.6%, 20% and 13.3% respectively; from Harey Bharey nuggets of CP meat were 13.3%, 6.6%, 13.3%, 20%, 13.3%, 20%, 33.3%, 13.3% and 13.3% respectively and that from the chicken tempura of CP meat were 13.3%, 13.3%, 20%, 20%, 13.3%, 6.6%, 40%, 33.3% and 13.3% respectively. The result were in concurrence with the findings reported [34] that contamination of Staphylococcus species, E. coli and Salmonella species in thigh muscle was more than in breast muscle irrespective of the processing condition. This higher prevalence of bacteria in thigh muscles as compared to the breast muscles might be due to their proximity to the evisceration point and maximal handling of the thigh region during the dressing operations.

In other study, 14 bacterial species have been isolated from the different frozen chicken parts which were Klebsiella aerogenes, Pseudomonas putida, S. aureus, Salmonella species, S. epidermidis, Proteus vulgaris, E. coli, Bacillus cereus, Corynebacterium species, Flavobacterium, Alcaligene species, Micrococcus species, Pseudomonas capacia, and B. subtilis [23] while 9 bacterial species were isolated in this study.

In the present study, the enteric organisms isolated are source of faecal contamination. These organisms are the sources of diarrhoea and or gastro-intestinal disturbances to both children and adults when consumed and may lead to food intoxication [42-44]. Similarly, B. cereus is a Gram-positive spore forming rod and food borne pathogen responsible for emetic and diarrheal syndrome due to the production of enterotoxins which can tolerate harsh conditions [45].

For cooking and storage of foods, adequate temperature is important for minimizing bacterial growth and the food may act as incubator for pathogenic bacteria which cannot maintain the safety temperature zone whether the food is raw, partially cooked or fully cooked [46,47].

In the present studies fungal contamination in the chicken meat samples were checked and it is found that most prevalent fungus was yeast isolated from 38.3% samples followed by mould Aspergillus niger from 16.1% samples, Aspergillus fumigatus from 14.4% samples Aspergillus flavus from 12.7% samples Penicillium chrysogenum from 12.2% samples Rhizopus stolonifer from 10% samples Fusarium equiseti from 9.4% samples and least prevalent mould was Fusarium avenaceum isolated from 0nly 3.3% samples. There were 22.2% samples found which had no fungal contamination.

From the three categories of the chicken meat it was found that in the RU meat the prevalence of A. niger, A. fumigatus, A. flavus, P. chrysogenum, R. stolonifer, F. equiseti, F. avenaceum and Yeast were 23.3%, 16.6%, 11.6%, 15%, 18.3%, 16.6%, 0% and 43.3% respectively; in the RP meat were 16.6%, 16.6%, 20%, 13.3%, 6.6%, 10%, 10% and 28.3% respectively and that in the CP meat were 8.3%, 10%, 6.6%, 8.3%, 5%, 1.6%, 0% and 43.3% respectively.

Contrary to the findings of the present study, Prevalence of Yeast in Luncheon and Shawerma were 65% and 70% respectively and that of moulds were 50% and 65% respectively [24]. In another study, Saccharomyces cerevisiae, Rhodotorula and Candida species are the yeast isolates from the chicken stored under different conditions [23]. Another study reported that presence of Rhizopus species causes an elevation of pH beyond safety level of 4.6 which makes the environment favorable for pathogenic bacteria [48].

Owing to the production of different types of metabolites by the microbes, contaminated chicken meat could be fatal. For instance, Aspergillus species isolate from the sample might have been introducing as spores from the environment and the Aspergillus are known to produce Aflatoxin [49]. Fungal contaminations in chicken usually took place due to mishandling, improper processing, washing with polluted water, packaging, deboning, and may be due to flies, dust, equipment's, workers and fluctuation of temperature during storage and transportation [50,51].

CONCLUSION

It is concluded from the study that 9 different species of both Gram positive and Gram-negative bacteria and 8 types of fungal species including pathogenic strains contaminate chicken meat at a higher lever and unacceptably contaminated for human consumption. However, it might be due to the poor sanitary environment of the slaughtering place and poor personal hygiene of the workers handling the chicken meat during processing and packaging. It is also concluded that the thigh muscle of chicken meat

has more bacterial load than breast part which suggests it to be more frequently used while product processing.

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